Evaluation of the Rapid ID 32A system for the identification of the *Bacteroides fragilis* group

Anna King and Ian Phillips

Department of Microbiology, UMDS, St Thomas' Campus, London, UK

Objective: To evaluate the use of a rapid identification system, Rapid ID 32A (bioMérieux), for the identification of clinically important species in the *B. fragilis* group.

Methods: The use of Rapid ID 32A was validated on 249 clinical isolates, all of which were tested by conventional techniques, and in selected instances API 20A. Rapid ID 32A (and API 20A as appropriate) was then applied in a central laboratory to the identification of 1289 *B. fragilis* group clinical isolates from 22 laboratories in 15 European countries.

Results: Improvements in the initial database permitted the accurate identification of isolates of *B. fragilis*, *B. thetaiotaomicron* and *B. vulgatus*, but further tests, especially for catalase production, were required to distinguish between *B. ovatus* and *B. uniformis*, while an identification of *B. distasonis* could be accepted only after careful review of results. There were too few isolates of *B. caccae*, *B. merdae* and *B. stercoris* for us to reach satisfactory conclusions, but further tests are clearly necessary.

Conclusions: The study emphasizes the importance of including sufficient numbers of isolates of different species in the validation of identification methods. Rapid ID 32A is a reliable system for the identification of the common species in the *B. fragilis* group, especially *B.fragilis* and *B. thetaiotaomicron*.

Key words: *Bacteroides fragilis* group, Rapid ID 32A, identification

INTRODUCTION

Conventional methods for the identification of anaerobic bacteria are time-consuming and expensive to prepare, and not commercially available in many parts of the world. For this reason the many kit tests that have been developed and marketed have been very much welcomed. These include miniaturized versions of conventional biochemical tests such as API 20A and Minitek, and, more recently, rapid tests based on preformed enzymes, which give results within 4 h, e.g. RapID ANA II and Anident. It is a characteristic of most kits that they can be used for the identification of all types of anaerobe likely to be isolated from clinical material. The performance of many of them has been assessed on collections of clinical isolates that have often contained small numbers of individual species. For example Appelbaum et al. [1] included 165 clinical isolates and 32 stock cultures representing 37 different species in their comparison of three methods for anaerobe identification; for 14 of these species there was only one isolate included, and for a further nine species there were only two or three isolates.

As part of a study of the antimicrobial susceptibility of the *Bacteroides fragilis* group [2] we collected large numbers of each of the clinically important species from many parts of Europe, avoiding isolates from feces or from clearly fecally contaminated specimens. We used this collection to assess the performance of a newly available rapid identification kit, Rapid ID 32A (bioMérieux), with the specific intention of assessing the effect of the inclusion of significant numbers of less common clinically important species.

Corresponding author and reprint requests:

Anna King, Department of Microbiology, UMDS, St Thomas' Campus, London, SE1 7EH, UK

Tel: (44) 171 928 9292 Fax: (44) 171 928 0730

Accepted 14 April 1996

MATERIALS AND METHODS

Organisms

A maximum of 100 consecutive isolates belonging to the *B. fragilis* group, from clinically infected sites, was

collected in each of 22 laboratories in 15 European countries and sent to St Thomas' Hospital, London. On arrival the organisms were subcultured on Columbia agar (Oxoid CM331) containing 7% horse blood, purified from mixed cultures where necessary, and stored in 7% glycerol broth at −70 °C.

The isolates were identified by the sending laboratories, either as belonging to the *B. fragilis* group or to species level within the group, by a variety of methods (API 20A, Minitek or conventional biochemical tests, but not Rapid ID 32A).

Identification methods

All isolates were tested at St Thomas' Hospital in Rapid ID 32A, a 4-h identification system developed by bioMérieux and consisting of 29 tests for preformed enzymes—urease, arginine dihydrolase, alkaline phosphatase, glutamic acid decarboxylase, 12 amino acid arylamidases (alanine, arginine, glycine, glutamyl-glutamic acid, histidine, leucine, leucylglycine, phenylalanine, proline, pyroglutamic acid, serine and tyrosine), nine carbohydrate-degradation enzymes (alpha-arabinosidase, alpha-fucosidase, alpha- and beta-galactosidase, beta-galactosidase-6-phosphate, alpha- and beta-glucosidase, beta-glucuronidase and beta-*N*-acetyl-glucosaminidase), two carbohydrate-fermentation enzymes (mannose and raffinose), indole production and nitrate reduction.

Organisms were subcultured from the frozen broth on Columbia blood agar and incubated overnight in an atmosphere of 80% nitrogen, 10% hydrogen and 10% carbon dioxide. In accordance with instructions, a heavy suspension, equivalent in turbidity to 4 McFarland units, was prepared in 2 mL of sterile distilled water and 55 μL inoculated into each cupule. The urease cupule was overlaid with mineral oil and the strips were covered and incubated in air at 37 °C for 4 h. Appropriate reagents were added to nitrate, indole, alkaline phosphatase and the amino acid arylamidase cupules to develop the reactions, and the tests were read after 5 min. Results were recorded and a 10-digit profile number generated according to the protocol provided. The identity of the isolates was determined from an analytic profile index (version 1.0), provided by the manufacturer, consisting of the first eight digits of the profile number. Where differentiation between two species was not possible, the reactions of tests included in the final two of the 10 digits of the profile number were checked against a table of expected results to separate them if possible. Profile numbers not included in the index were sent to the manufacturers for computer analysis.

Isolates that were not identified to species level by this system, or that identified as *B. distasonis* but were arabinose negative and glycine arylamidase positive, were tested in the API 20A system. If the identification from Rapid ID 32A differed from that of the sending laboratory, isolates were also tested in API 20A. In addition, isolates that were identified as species unexpected in uncontaminated clinical specimens (*B. caccae/loesheii/merdae/stercoris*) were also tested in the API 20A system.

Catalase production was determined with ID color Catalase reagent (bioMérieux) dropped directly on the culture plate and scored as positive only if oxygen bubbles were released within 5 s and before the reaction of a drop placed on an uninoculated part of the agar. The identification of isolates was accepted on the basis of Rapid ID 32A results, with catalase if necessary, if the results agreed with those of the sender. For the remainder, the results of the API 20A were also included.

Towards the end of the study an updated version of the profile index for Rapid ID 32A (version 3.0) was provided, in a computerized form, by bioMérieux and all profile numbers were re-analyzed by the same criteria.

Validation of identification method

The validity of the use of Rapid ID 32A followed, where no identification was obtained, by API 20A was investigated by comparing results of identification with those of three of the participating laboratories which had identified their isolates by conventional biochemical tests. All three laboratories tested for growth in 20% bile, for indole production and for fermentation of from five to 11 carbohydrates with glucose, arabinose, trehalose and salicin in common. In addition, laboratory 1 tested for catalase production, laboratory 2 for nitrate reduction and laboratory 3 for aesculin and starch hydrolysis. Isolates for which the study identification differed from that obtained by conventional methods were retested in Rapid ID 32A, which was found to be reproducible, and resubmitted to one of the three laboratories for repeat analysis.

RESULTS

Validation study

There were 249 isolates from the three laboratories that used standard identification methods included in the validation. These were finally identified, by conventional methods, as 147 isolates of *B. fragilis*, 32 *B. uniformis*, 25 *B. thetaiotaomicron*, 15 *B. vulgatus*, 14 *B. ovatus*, 11 *B. distasonis*, two *B. stercoris*, one *B. caccae* and 2 unnamed *B. fragilis* group members. The numbers of isolates received from each of the three laboratories and those for which there was agreement or discrepancy

Table 1 Discrepancies between standard and study methods of identification of *B. fragilis* group organisms in validation study

		Difference in:			
	Number of isolates	Number not in agreement	Indole production	Trehalose fermentation	Interpretation of results
Laboratory 1	106	1	0	1	0
Laboratory 2	96	33	19	9	5
Laboratory 3	47	25	20	5	0

between conventional and study methods are shown in Table 1. There was agreement in identification for all but one isolate from laboratory 1, and the difference was in the interpretation of trehalose fermentation which was positive in prereduced anaerobically sterilized (PRAS) medium and negative in API 20A, and this resulted in a different allocation to the closely related species *B. ovatus* and *B. uniformis*, a difference that remained after retesting. Differences for 19 of the 33 isolates from laboratory 2 were attributable to discrepancies in results for indole production, and when these were retested all but one were in agreement with the Rapid ID 32A results (Table 1). A further nine isolates had differences in the results for trehalose fermentation, seven of which were positive in PRAS and negative in API 20A, and they were thus assigned to different but closely related species. For the remaining five isolates there were no differences in results of biochemical tests; two of these were the newly described species *B. caccae* and *B. stercoris* and the remaining three had atypical biochemical results and were assigned to different closely related species. Among the isolates from laboratory 3, for 20 of the 25 for which the identification did not agree, the discrepancies were attributable to differences in results for indole production, and when these were retested all agreed with the results from Rapid ID 32A. The remaining five isolates were closely related indole-positive species, and differences were again attributable to interpretation of trehalose fermentation.

It was concluded that identifications based on the three local variations of conventional methods of anaerobe identification were the same as those obtained from Rapid ID 32A plus API 20A, once allowance had been made for the facts that indole results for Rapid ID 32A were always correct, and the detection of weak trehalose fermentation from API 20A was sometimes unreliable.

Definitive study

A total of 1422 cultures was received from the 22 laboratories, of which 1289 cultures yielded isolates in the *B. fragilis* group. The distribution of species as finally determined on the basis of all investigations is listed in Table 2. Almost all the isolates were allocated to recognized species, but there were eight, belonging to the group, that were not identified to species level despite repeated biochemical tests.

There were 88 different eight-digit profiles and 169 different 10-digit profiles generated by the use of

Table 2 Results of Rapid ID 32A with eight-digit profiles in comparison with definitive identity for 1289 *B. fragilis* group isolates

		Profile index version 1				Profile index version 3			
	Number of isolates	Correct species	Genus	Incorrect species	No valid identification	Correct species	Genus	Incorrect species	No valid Identification
B. fragilis	736	83 (11)	566 (77)	44	43	669 (91)	57 (8)	5	5
B. distasonis	48	45 (94)	0	0	3	40 (83)	7 (15)	0	1
B. vulgatus	71	44 (62)	9 (13)	10	8	47 (66)	14 (20)	8	2
B. thetaiotaomicron	193	103 (53)	11 (6)	2	77	177 (92)	13 (7)	2	1
B. ovatus	112	0	107 (95)	0	6	0	109 (97)	0	3
B. uniformis	106	0	88 (83)	2	15	0	101 (95)	3	2
B. caccae	8	4	1	0	3	5	2	0	1
B. merdae	1	0	0	1	0	0	1	0	0
B. stercoris	6	1	0	0	5	2	3	0	1
B. fragilis group	8	0	1	3	4	0	6	1	1
Total	1289	280 (22)	783 (60)	62 (5)	164 (13)	940 (73)	313 (24)	19 (1)	17 (1)

Numbers in parentheses are percentages.

Table 3 The sequence of investigation for identification of 1289 *B. fragilis* group isolates

	Profile index version 1				Profile index version 3			
	Correct species	Genus	Incorrect species	No valid identification	Correct species	Genus	Incorrect species	No valid identification
Eight digit profile	280 (22)	783	62	164	940 (73)	313	19	17
+catalase	844 (65)	219	62	164	949 (74)	304	19	17
Ten-digit profile	861 (67)	228	65	135	965 (75)	286	25	13
+API 20A with or without catalase	1281 (99)	0	0	8	1281 (99)	0	0	8

Rapid ID 32A. The sequence of investigations that led to the definitive identification of the isolates with version 1 of the profile index is shown in Table 3.

Eight-digit profile: version 1

With the use of the eight-digit profiles, 342 isolates were assigned species names. Only 280 (22% of the total) were eventually deemed correct: for 245 of them there were no atypical biochemical reactions and the identification agreed with that of the sending laboratory when given, while the remaining 35 were confirmed by API 20A because the identification differed from that of the sending laboratory.

A further 783 (61%) isolates were identified to genus level, usually with the choice of two closely related species given (Table 3). Of the 783, 565 had the same eight-digit profile, with a choice between *B. fragilis* and *B. caccae*, and these two species were then differentiated on the basis of catalase production. All 565 were catalase positive and thus identified as *B. fragilis*. However, for 27 of these catalase-positive isolates the identification differed from that of the sending laboratory and the identification was therefore confirmed in API 20A. For a further 195 of the 783 isolates, all with the same eight-digit profile, the profile was unable to distinguish between *B. uniformis* and *B. ovatus*, and all of these were tested in API 20A and for catalase production. Six were identified as *B. thetaiotaomicron*, 83 as *B. uniformis* and 106 as *B. ovatus*. The remaining 23 of the 783 isolates that were identified to genus level were tested in API 20A as there was no simple method of differentiating the species suggested by Rapid ID 32A.

Of the 62 organisms that were incorrectly identified in Rapid ID 32A, 35 of the 44 *B. fragilis* (34 with the same eight-digit profile), eight of the 10 *B. vulgatus* (five profiles) and the one *B. merdae* were misidentified as *B. distasonis* (Table 4), as were two of the isolates that we were eventually unable to speciate. There were 14 isolates incorrectly identified as species

Table 4 Incorrect identification of isolates with eight-digit profiles

Final identification	Number of isolates	Incorrect identification version 1	Number of isolates	Incorrect identification version 3
B. fragilis	44	35 *B. distasonis* 3 *B. caccae* 2 *B. merdae* 4 *B. loescheii*	5	1 *B. distasonis* 1 *B. caccae* 2 *B. merdae* 1 *B. thetaiotaomicron*
B. vulgatus	10	8 *B. distasonis* 1 *B. caccae* 1 *B. merdae*	8	4 *B. distasonis* 3 *B. caccae* 1 *B. merdae*
B. thetaiotaomicron	2	1 *B. ovatus* 1 *B. stercoris*	2	1 *B. caccae* 1 *B. stercoris*
B. uniformis	2	1 *B. thetaiotaomicron* 1 *B. stercoris*	3	2 *B. thetaiotaomicron* 1 *B. stercoris*
B. merdae	1	1 *B. distasonis*		
B. fragilis group	3	2 *B. distasonis* 1 *B. ovatus*	1	1 *B. caccae*

such as *B. caccae*, *B. merdae* or *B. stercoris* that were not expected to be isolated in the study, and two of the closely related indole-positive isolates were misidentified, one *B. thetaiotaomicron* as *B. ovatus* and one *B. uniformis* as *B. thetaiotaomicron*.

Ten-digit profile: version 1

There were 164 isolates for which the eight-digit profile (49 different profiles) was not in the index book (Table 3), so all these were tested in API 20A. The 10-digit profiles were sent to bioMérieux for computer-assisted analysis: only 20 of the 164 were returned identified to species level, of which 17 were deemed correct. The three misidentified isolates, which were eventually identified as different species, all had the same 10-digit profile and were identified as *B. ovatus* by the computer. A further nine of the 164 isolates, identified to genus level, were indole positive and had the same 10-digit profile. Seventy-seven of the 164 isolates, for which there was no valid identification, were *B. thetaiotaomicron*: 35 of these had the same eight-digit profile (four 10-digit profiles) and a further 21 had another eight-digit profile (five 10-digit profiles). Only six of these 77 isolates were identified to the correct species with computer assistance but for a further 50 of the 77 isolates the species listed were a choice between *B. thetaiotaomicron* and *B. caccae* and these could be differentiated by testing for catalase production.

Eight-digit profile: version 3

With the updated version 3 of the profile index, 959 of the 1289 isolates were assigned species names, and 940 (73% of the total 1289 isolates) of these were correctly identified (Table 3). A further 313 of the 1289 were identified to genus level, and nine of these could be speciated by the addition of catalase. Nineteen of the 1289 isolates were incorrectly speciated and there was no valid identification for 17 isolates.

Ten-digit profile: version 3

Only 19 of the 304 isolates identified to genus level, but unspeciated by the eight-digit profile, were assigned species names when the 10-digit profile was used and, 11 of these were correct. A further eight of these 304 had no valid identification, and the remaining 277 were unchanged; 210 of these 277 belonged to the two closely related species *B. ovatus* and *B. uniformis*. Of the 19 isolates incorrectly speciated with the 8-digit profile, three were identified to genus level and the rest were unchanged with the 10-digit profile: four *B. vulgatus* and one *B. fragilis* were misidentified as *B. distasonis*, and two of the *B. uniformis* and one *B. fragilis* were misidentified as *B. thetaiotaomicron*. Eleven isolates, all of which were catalase positive, were misidentified as *B. caccae*, *B. merdae* or *B. stercoris*. For the 17 isolates with no valid identification, six were speciated, five of these were correct, six were identified to genus level and five remained unchanged.

For most of the species the majority of isolates were characterized by relatively few eight-digit profiles, but there were many profiles for which there was only one isolate.

There were also 18 eight-digit profiles that were shared by isolates from more than one species and for the majority of these the addition of the extra tests to create the 10-digit profile was not helpful in distinguishing, particularly, the indole-positive species. The two closely related species *B. ovatus* and *B. uniformis* could usually be separated by results of API 20A and catalase production, but there was a small number for which results from a combination of tests from both Rapid ID 32A and API 20A had to be assessed before final speciation was possible.

DISCUSSION

Conventional biochemical tests are considered the standard against which all other methods are assessed. However, there is a need for skill and experience in both the manufacture of the media and interpretation of results. In many parts of the world the media must be manufactured within the laboratory and hence are used in only a small number of laboratories with a specialist interest in anaerobic bacteria—in our study three out of 22 laboratories.

Several studies [1,3–5] have reported good correlation between API 20A and conventional tests for the *B. fragilis* group and thus, after validation of the method in our own laboratory, we used API 20A in this study as our comparative method for identification. Fermentation tests in API 20A often have weak reactions and hence may be difficult to interpret but with experience the system has proved generally valid.

Ideally, the results of the Rapid ID 32A should have been compared with conventional tests for all isolates but this was physically impossible because of the large numbers and the lack of commercially available PRAS tests in the UK. We therefore compared the results of Rapid ID 32A with those of three laboratories that had identified their isolates by conventional methods in media prepared in their own experienced laboratories. This highlighted the fact that conventional tests can be regarded as the standard only if the media are correctly prepared and strict quality control is adhered to. In this study the discrepancies in the results for indole production, particularly those from laboratory 3, which reported many false-negative results, were possibly due to incorrect pH of the medium [6],

or to an insufficient tryptophan content [7], or nitrite may have been present in the medium before inoculation [8,9]. It is difficult to account for differences where the conventional tests were positive for indole and negative in both Rapid ID 32A and API 20A. If several isolates were grown on plates for the spot indole test, the indole might have diffused from positive isolates to negative isolates and resulted in false-positive results [9], and it is at least possible that some of the cultures were mixed and we chose the indole-negative isolate when the cultures were purified. Whatever the explanation, these differences were eradicated when the isolates tested in Rapid ID 32A and API 20A were resubmitted to be tested again by conventional tests. Our conclusion was that indole results from both of the kit tests were as reliable as those from the best laboratory that used standard methods.

The differences in results for trehalose fermentation were probably due to small decreases in pH which were detected in PRAS media but not in API 20A because of the difference in pH measurement—a pH meter for PRAS media and bromothymol blue indicator for API 20A. The isolates with the same results for biochemical tests for both PRAS media and Rapid ID 32A and API 20A but assigned to different species highlight the problems, particularly for closely related species, associated with choosing a small number of tests in PRAS media to identify isolates with atypical results in one or two tests, and the advantage of testing a wider range of tests more easily in the commercial systems.

The availability of a 4-h aerobic identification system for anaerobic bacteria has the obvious advantages of speed and space saving, particularly for a large study such as this. Although there was little information available on the new Rapid ID 32A system [10,11] (unpublished evaluation studies supplied by the manufacturer), the results were sufficiently encouraging for us to use the system for this study.

Reading of the reactions in Rapid ID 32A may be difficult, particularly the aminopeptidase reactions, with some reactions being much stronger than others and some organisms giving weak reactions throughout, so that reactions interpreted as doubtful for one organism may be positive for another. This meant that the system was more difficult to use than had originally been anticipated, and although all the organisms were tested by one laboratory scientist there is no doubt that for some of the organisms the interpretation of reactions may have been incorrect. Nevertheless, for the majority of the isolates there was no difficulty in interpretation once the criteria were established.

As all the isolates were in the *B. fragilis* group, inherent problems with version 1 of the profile index for Rapid ID 32A soon became obvious. The most striking of these was the misidentification of some organisms, mostly those finally identified as *B. fragilis*, as *B. distasonis*. The two tests responsible for this error were alpha-arabinosidase and glycine arylamidase, both of which are usually positive for *B. distasonis* and negative for *B. fragilis*. Thus even if the organisms were alpha-arabinosidase negative, they were identified as *B. distasonis* if the glycine arylamidase was positive. Thirty-five of the 736 (5%) isolates of the *B. fragilis* in this study were glycine arylamidase positive, and the identification was confirmed with API 20A, in which differences in results for trehalose and salicin were more discriminatory. Eight (11%) of the *B. vulgatus* isolates were also misidentified, and half of these were wrongly speciated as *B. distasonis*. This again was because of positive results in tests which were expected to be negative from the database.

Over half the isolates in this study were *B. fragilis*, and although only 11% of these were identified by version 1 of the profile index alone, a further 77% were identified to genus level and could be differentiated from *B. caccae*, the alternative choice given, by testing for catalase production [12].

Version 1 of the Rapid ID 32A profile index was equally unsuccessful in identifying the indole-positive isolates to species level, with the exception of *B. thetaiotaomicron*, for which 53% were correctly identified without further tests. The choice between *B. caccae* and *B. thetaiotaomicron* from the 10-digit profile for 50 further isolates was a surprising one, since all were indole positive and *B. caccae* is indole negative. Although the identification of these isolates was confirmed in API 20A, the two species could in fact be differentiated on the basis of catalase production.

The introduction of the updated version 3 of the profile index made a very significant difference to the identification rate, particularly for *B. fragilis*, for which it increased from 11% to 91%, and for *B. thetaiotaomicron*, for which it increased from 53% to 92%. The number of isolates wrongly speciated also decreased, but there were still four isolates of *B. vulgatus* misidentified as *B. distasonis*.

Neither version of the Rapid ID 32A profile index was able to distinguish between *B. ovatus* and *B. uniformis*, although 89% of isolates that were finally identified as one or other of these two species were identified to genus level, with a choice between the two species given as the most likely identification. The extra tests quoted in the profile index for distinguishing between these two species included catalase with percentage positive of 92% for *B. ovatus* and 0% for *B. uniformis*. These figures are at variance with other reference methods such as the VPI manual [8], which

quotes both species as negative with occasional positive strains. Methodology has also been shown to influence the results of tests for catalase production [13], but although these workers agree with 0% for *B. uniformis*, the results for *B. ovatus* varied from 57 to 80%, depending on the method used. On the basis of these conflicting results we tested all strains of these two species in API 20E as well as testing for catalase production, and by this method we found, when final identification was reached, that 77% of *B. ovatus* and 28% of *B. uniformis* were catalase positive. The differentiation of these two closely related species can be difficult even with conventional biochemical tests, and there was a small but significant number of isolates in this group for which we had to assess the strength of some reactions in both Rapid ID 32A and API 20A to speciate them.

Since we undertook this study there have been several reports on the use of the Rapid ID 32A system [14–16], each of which included less than 100 isolates in the *B. fragilis* group. None of the studies included more than 11 strains each of species other than *B. fragilis* and *B. thetaiotaomicron*, and none had more than three strains of *B. caccae*, *B. merdae* or *B. stercoris*. In all these studies, in which version 1 of the profile index was presumably used, the percentage of isolates correctly identified without the use of extra tests was higher than in our study, but all had similar problems discriminating between closely related species, particularly *B. ovatus* and *B. uniformis*. In one study, which tested only isolates in the *B. fragilis* group [14], 23% of the isolates were culture collection strains (ATCC, NCTC or VPI) which, presumably, are biochemically typical and could account for the apparently improved performance of the system. We agree with Kitch and Appelbaum [15] that, with version 1 of the profile index, catalase was a required additional test allowing the proportion of correctly identified isolates to increase. The number of isolates included in the preparation of the data base for version 1 is not known, but was presumably small since this is a new system. Expansion of the data base to produce version 3 clearly increased the overall performance of the system for the *B. fragilis* group of organisms.

Undoubtedly, when large numbers of isolates in a group are tested biochemically, aberrant strains will be included, and it is not surprising that we had some isolates that were identified incorrectly or for which the profile was not included in the profile index. From the results of this study we conclude that, with the present Rapid ID 32A database, identification of isolates as *B. fragilis*, *B. thetaiotaomicron* or *B. vulgatus* can be confidently accepted without further testing. Isolates identified as *B. distasonis* can be accepted only after careful review of the test results and even then with caution. The system is unable to distinguish between *B. ovatus* and *B. uniformis* and further tests including, but not exclusively, catalase must be done for correct speciation. Although there were, as we expected, only small numbers of isolates in this study that were finally identified as *B. caccae*, *B. merdae* or *B. stercoris*, and thus we have little knowledge of the validity of the database profile in relation to these three species, an unacceptable number of isolates were misidentified on the basis of kit tests as belonging to one of the three, and therefore we suggest that such identifications should not be accepted without further testing.

We feel that Rapid ID 32A would be useful for the identification of this group of organisms in a diagnostic laboratory, as it performed well for the species most commonly isolated, *B. fragilis* and *B. thetaiotaomicron*. It does, however, require familiarity to interpret some reactions.

It remains to be seen how the Rapid ID 32A will perform with other species for which the database is still built on relatively small numbers of isolates. We have described the various steps in our study in some detail to demonstrate the problems that arose from an uncritical evaluation of reports that purport to validate identification systems. A study such as ours, ideally including results from well-validated conventional tests for all the isolates studied, should be performed for all species to include biochemically atypical isolates, to increase the size of the database and to validate the system before it can be used with complete confidence in clinical laboratories.

Acknowledgements

This study was supported by grants from the European Society for Clinical Microbiology and Infectious Diseases and from bioMérieux UK Limited. We would like to thank Mr D. Quinn and Mr H. Beatie for their invaluable help with the technical work, and Dr Brian Thomas and his staff at bioMérieux for their assistance in overcoming the problems of interpretation.

We also thank the following for providing strains: Prof. J.F. Acar, Paris, France; Dr F. Baquero, Madrid, Spain; Dr A. Bremmelgaard, Frederiksberg, Denmark; Prof. J.A. Garcia-Rodriguez, Salamanca, Spain; Prof. U. Höffler, Dusseldorf, Germany; Prof. T. Hofstad, Bergen, Norway; Dr H. Jousimies-Somer, Helsinki, Finland; Prof. N.J. Legakis, Athens, Greece; Prof. D.M. MacLaren, Amsterdam, Netherlands; Dr P. Maes, Oostende, Belgium; Dr G.J. Meijer-Severs, Groningen, Netherlands; Dr H. Mittermayer, Linz, Austria; Prof. C-E. Nord, Stockholm, Sweden; Prof. G. Panichi, Rome, Italy; Prof. G. Privitera, Milan, Italy; Dr A. Sedallian, Annecy, France; Prof. S.

Tabaqchali, London, UK; Prof. A. Torres Pereira, Lisbon, Portugal; Prof. U. Ullmann, Kiel, Germany; Prof. J. Wüst, Zurich, Switzerland; Prof. E. Yourassowsky, Brussels, Belgium; and we thank Dr Jousimies-Somer, Prof. Nord and Dr Meijer-Severs, who carried out conventional tests.

References

1. Appelbaum PC, Kaufmann CS, Keifer JC, Venbrux HJ. Comparison of three methods for anaerobe identification. J Clin Microbiol 1983; 18: 614–21.
2. Phillips I, King A, Nord CE, Hoffstedt B. Antibiotic sensitivity of the *Bacteroides fragilis* group in Europe. Eur J Clin Microbiol Infect Dis 1992; 11: 292–304.
3. Karachewski NO, Busch EL, Wells CL. Comparison of PRAS II, RapID ANA, and API 20A systems for identification of anaerobic bacteria. J Clin Microbiol 1985; 21: 122–6.
4. Moore HR, Sutter VL, Finegold SM. Comparison of three procedures for biochemical testing of anaerobic bacteria. J Clin Microbiol 1975; 1: 15–24.
5. Nord CE, Dablback A, Wadstrom T. Evaluation of a test kit for identification of anaerobic bacteria. Med Microbiol Immunol 1973; 161: 239–42.
6. Höhne C, Hübner G, Sandow D. Investigations of methodical problems for detecting indole production by anaerobic pathogens. Int J Microbiol Hyg 1987; 265: 314–22.
7. Sutter VL, Carter WT. Evaluation of media and reagents for indole-spot tests in anaerobic bacteriology. Am J Clin Pathol 1972; 58: 335–8.
8. Holdeman LV, Cato EP, Moore WEC. Anaerobe laboratory manual, 4th edn. Blacksburg, Virginia: Virginia Polytechnic Institute and State University, 1977: 31–7.
9. Summanen P, Baron EJ, Citron DM, Strong CA, Wexler HM, Finegold SM. Appendix B. In: Wadsworth anaerobic bacteriology manual, 5th edn. Belmont Ca: Star Publishing Company, 1993: 153.
10. Murdoch DA, Mitchelmore IJ, Tabaqchali S. Identification of Gram-positive anaerobic cocci by use of systems for detecting pre-formed enzymes. J Med Microbiol 1988; 25: 289–93.
11. van Winkelhoff AJ, Clement M, de Graaff J. Rapid characterization of oral and nonoral pigmented *Bacteroides* species with ATB Anaerobes ID system. J Clin Microbiol 1988; 26: 1063–5.
12. Johnson JL, Moore WEC, Moore LVH. *Bacteroides caccae* sp.nov., *Bacteroides merdae* sp.nov., and *Bacteroides stercoris* sp.nov. isolated from human feces. Int J Syst Bacteriol 1986; 36: 499–501.
13. Wilkins TD, Wagner DL, Veltri VJ Jr, Gregory EM. Factors affecting production of catalase by *Bacteroides*. J Clin Microbiol 1978; 8: 553–7.
14. Jenkins SA, Drucker DB, Keeney MGL, Ganguli LA. Evaluation of the Rapid ID 32A system for the identification of *Bacteroides fragilis* and related organisms. J Appl Bacteriol 1991; 71: 360–5.
15. Kitch TT, Appelbaum PC. Accuracy and reproducibility of the 4-hour ATB 32A method for anaerobic identification. J Clin Microbiol 1989; 27: 2509–13.
16. Looney WJ, Gallusser AJC, Modde HK. Evaluation of the ATB 32A system for identification of anaerobic bacteria isolated from clinical specimens. J Clin Microbiol 1990; 28: 1519–24.